Development of a LAMP/Cas12a assay to specifically detect the California strain of resistance breaking (RB) - Tomato spotted wilt virus (TSWV) in tomato

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Rapid and accurate detection of strains of plant-pathogenic viruses is critical for disease outbreak surveillance. In California (CA), the widespread use of tomato cultivars with the *Sw-5* tospovirus resistance gene has led to the occurrence and spread of an RB-TSWV strain. The objectives of our study were to 1) compare disease progress and impact on plant growth of the RB to a wild type (non-RB) strain on tomato with (cv. 'Mountain Merit') and without (cv. 'Mountain Fresh Plus') the *Sw-5* gene; 2) determine incidence of detection of RB and non-RB TSWV using microneedle RNA extractions and LAMP; and 3) develop a rapid LAMP/Cas12a assay for detection of the TSWV *NSm* mutation in the RB strain. Susceptible plants showed 15% - 25% stunting when inoculated with either strain compared to non-infected controls. *Sw-5* plants had little disease when inoculated with non-RB but exhibited severe stunting (>50%) when inoculated with RB. The detection of positive LAMP reactions from susceptible tomatoes was higher in non-RB than RB over time. The RB strain remained detectable in susceptible tomato over the 14 days, while non-RB was undetectable by LAMP tests in resistant tomato. We developed a two-step LAMP/Cas12a protocol that differentiates the two strains within one hour that includes colorimetric LAMP followed by Cas12a. Our methods were validated with plants under chamber and field conditions, an indication that this method shows promise for detection of RB-TSWV in the field.